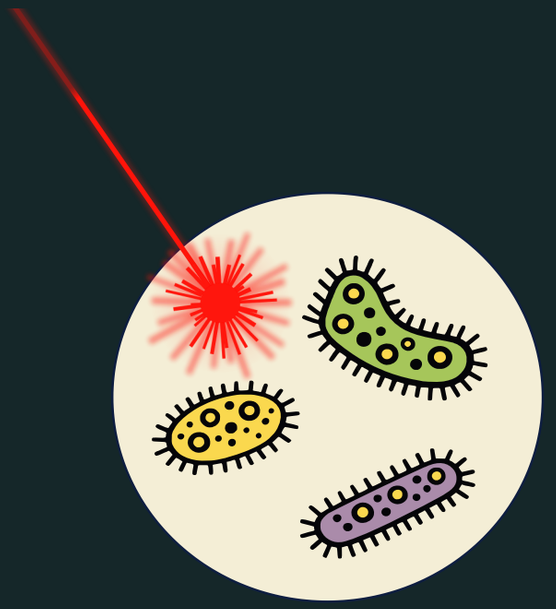
Raman bacteria identification



From colony to answer within one hour

With Lightnovo RG and miniRaman microscopes

97 % — **Species identification after full cultivation**

15 species · 66 of 68 colonies correctly identified

Single-device classification

INTRODUCTION

Fast identification of the pathogen is the first step of targeted antibiotic therapy. The gold standard, culture plus MALDI-TOF MS, needs 18–48 h of growth and an instrument of 180–300 k€ [1]; until then patients get empirical broad-spectrum antibiotics. Hospital infections cause more than 90,000 deaths per year in Europe [2].

Raman spectroscopy reads the molecular fingerprint of a cell (proteins, lipids, nucleic acids) label-free, without reagents. With machine learning it identifies bacteria at species level with more than 96 % accuracy [3].

High-throughput optics and Light Compression technology allowed Lightnovo's miniaturized Raman spectrometers to reach the accuracy of research-grade spectrometers at 0.2 s per spectrum instead of 10 × 1 s [4].

This Application Note shows five use cases with Lightnovo Raman microscopes: 15 species identified with 97 % accuracy after full cultivation, up to 49 samples per target plate measured automatically, bacterial growth followed inside the instrument, and two culture-free screens: infected vs immunological wounds (91 %) and infected urine (81 %).

01	02	03	04	05
97 %	49	54 min	91 %	81 %
species accuracy after cultivation	samples per target plate, automated	to visible growth inside the instrument	wound-map accuracy, no culture	urine infection identification, no culture

THE MEASUREMENT PLATFORM

Two systems. One workflow

a b c



Figure 1. Lightnovo Raman systems. (a) RG Raman microscope (Rgm). (b) miniRaman microscope (mRm). (c) 49-well target plate in its holder.

Two Lightnovo Raman microscopes were used, with the same target plate, software and 100× NA 0.85 NIR-enhanced objective (Olympus LCPLN100XIR).

	RG Raman microscope (Rgm)	miniRaman microscope (mRm)
Spectrometer	RG Raman	miniRaman / miniRaman PRO
Laser wavelength	785 / 830 nm	785 / 830 nm*
Laser power	Up to 400 mW	miniRaman: 5–50 mW PRO: up to 400 mW*
Spectral range	785 nm: 70–2100 cm^{-1} 830 nm: 70–2400 cm^{-1}	miniRaman: 270–3100 cm^{-1} PRO 785: 100–3100 cm^{-1} PRO 830: 80–2400 cm^{-1}
Spectral resolution	3–5 cm^{-1}	miniRaman: 7–12 cm^{-1} PRO: 5–12 (785); 6–12 (830) cm^{-1}
Target-plate capacity	Standard 96-position MALDI-TOF plates	Automated measurement of 49-well plates

Available configurations; experimental settings are specified in the application sections. * miniRaman microscope: 830 nm requires PRO. PRO: up to 400 mW (multimode), or 70 mW (785 nm confocal).

Built-in calibration

A patented reference channel calibrates Raman shift and intensity in every measurement [4], so spectral libraries transfer between instruments and days.

Software

Miraspec: acquisition, mapping, preprocessing, PCA and classifiers.

In-instrument cultivation

Uses the external RG+ air temperature stabilizer.

KEY WORDS

Bacteria identification; Raman spectroscopy; clinical microbiology; Gram classification; machine learning; PCA; SVM; target plate; in-instrument cultivation; wound diagnostics; urinary tract infection

01 / IDENTIFICATION AFTER CULTIVATION

Species-level answers

97%

15 species identified
66 of 68 colonies correct

15 species identified with 97 % accuracy (66 of 68 colonies). Colonies grown 24 h at 37 °C were smeared onto the metal target plate and mapped at 785 nm (8 × 8 grid, 1–5 s per spectrum): 68 maps, 4107 spectra, 10 days over 5 months (Fig. 2). A PCA + SVM model was tested leave-one-map-out, with a majority vote per map.

All Gram-negative and yeast colonies were correct; both errors are Gram-positive species. Agar adds only 1.1 % to the spectrum, so the model reads the cells, not the medium. On an extended set (20 species, 234 maps) tested on unseen days, accuracy was 88–93 %, on par with published colony-level Raman results [5]. A convolutional neural network separates the same 15 classes (Fig. 3).

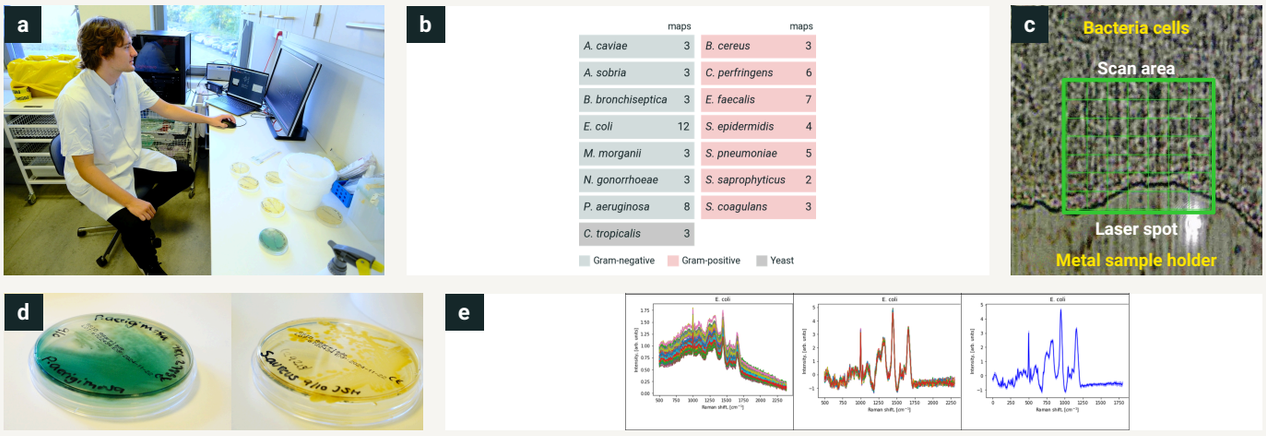


Figure 2. Data collection at Odense University Hospital. (a) Operator at the instrument. (b) The 15 species in the library, coloured by class, with the number of maps (colonies). (c) Colony smear on the metal sample holder with the 8 × 8 scan grid (64 spectra). (d) *P. aeruginosa* and *S. aureus* after 24 h on agar. (e) *E. coli* spectra: raw, preprocessed, mean ± SD.

TRUE \ PREDICTED	A. caviae	A. sobria	E. coli (asympt.)	B. cereus	B. bronchiseptica	C. perfringens	C. tropicalis	E. coli	E. faecalis	M. morganii	N. gonorrhoeae	P. aeruginosa	S. aureus	S. pneumoniae	S. coagulans
A. caviae	**0.96**	0.00	**0.04**	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
A. sobria	0.00	**0.95**	**0.05**	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
E. coli (asympt.)	0.00	0.00	**1.00**	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
B. cereus	0.00	0.00	**0.05**	**0.95**	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
B. bronchiseptica	0.00	0.00	**0.04**	0.00	**0.96**	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C. perfringens	0.00	0.00	**0.05**	0.00	0.00	**0.95**	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C. tropicalis	0.00	0.00	**0.05**	0.00	0.00	0.00	**0.95**	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
E. coli	0.00	0.00	**0.05**	0.00	0.00	0.00	0.00	**0.95**	0.00	0.00	0.00	0.00	0.00	0.00	0.00
E. faecalis	0.00	0.00	**0.05**	0.00	0.00	0.00	0.00	0.00	**0.95**	0.00	0.00	0.00	0.00	0.00	0.00
M. morganii	0.00	0.00	**0.05**	0.00	0.00	0.00	0.00	0.00	0.00	**0.95**	0.00	0.00	0.00	0.00	0.00
N. gonorrhoeae	0.00	0.00	**0.04**	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**0.96**	0.00	0.00	0.00	0.00
P. aeruginosa	0.00	0.00	**0.04**	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**0.96**	0.00	0.00	0.00
S. aureus	0.00	0.00	**0.05**	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**0.95**	0.00	0.00
S. pneumoniae	0.00	0.00	**0.05**	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**0.95**	0.00
S. coagulans	0.00	0.00	**0.04**	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**0.96**

Figure 3. Normalized confusion matrix of the convolutional neural network for the 15 classes, validation set: 0.95–1.00 of each class correctly identified (red); the remaining 0.04–0.05 is predicted as asymptomatic *E. coli*. Leave-one-map-out validation on a single device and a single agar plate for each bacterium.

02 / AUTOMATED IDENTIFICATION

From plate to species report

49

samples per miniRaman run
49 min at approximately 60 s per sample

The miniRaman microscope supports automated identification of up to 49 bacterial samples per run, while the RG microscope is compatible with standard 96-position (12 × 8) MALDI-TOF target plates.

Bacterial colonies are smeared directly onto steel target plates without additional sample preparation (Fig. 4a). Once the plate is loaded (Fig. 4b), Miraspec automatically locates sample positions, focuses, acquires Raman spectra, and identifies bacterial species using the reference library described in Section 01 (Fig. 4c).

The software generates a report with species identification and classification scores for each sample (Table 1). With approximately 60 s per sample, a full 49-sample plate can be analyzed in 49 min with minimal operator involvement.

a b c

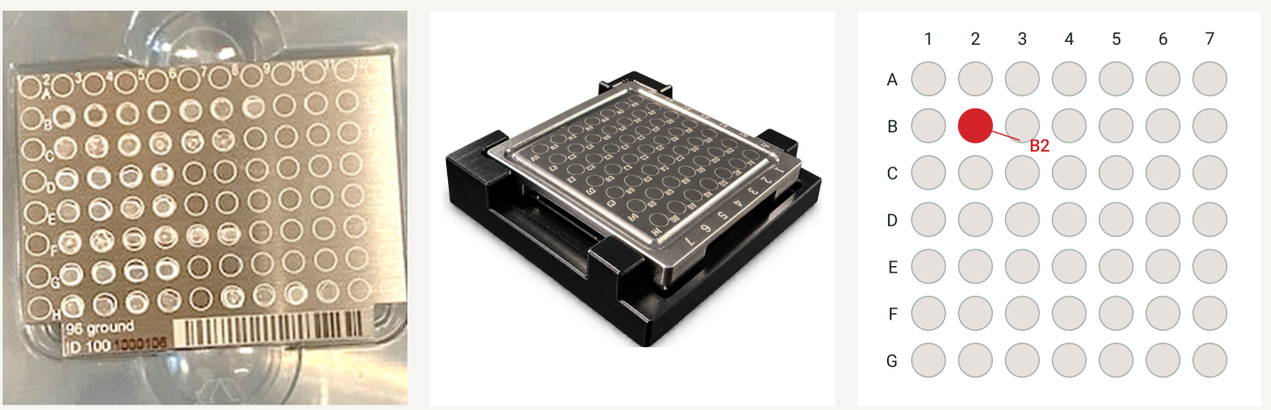


Figure 4. (a) Steel target plate with bacterial smears (MALDI-TOF format). (b) 49-well target plate in its holder. (c) Miraspec plate view with the active well (B2) highlighted.

Table 1. Illustrative Miraspec report excerpt: species and classification score per well. B2 matches the highlighted position in Fig. 4c.

Well	Identification	Score	Well	Identification	Score
A1	*E. coli*	0.97	B1	*E. faecalis*	0.93
A2	*K. pneumoniae*	0.95	**B2**	*B. cereus*	0.91
A3	*S. aureus*	0.98	B3	*C. tropicalis*	0.99
A4	*P. aeruginosa*	0.96	B4	*S. epidermidis*	0.94
…	…	…	…	…	…

03 / IN SITU CULTIVATION

Watch the colony grow

54 min

to visible *E. coli* growth
Early-stage species identification remains a next step.

Temperature-controlled cultivation inside the RG microscope enables bacterial growth to be monitored at the same location, demonstrating visible *E. coli* growth within 54 min and enabling Raman measurements of early-stage colonies.

E. coli was sparsely streaked onto an agar plate to obtain small bacterial colonies (Fig. 5a). The plate was placed directly inside the RG microscope, maintained at approximately 37 °C using the external RG+ air temperature stabilizer (Cube) (Fig. 6). This allowed bacterial growth to be monitored at the same location without transferring the plate between an incubator and the microscope.

Visible bacterial growth was observed after 54 min (Fig. 5b, c). Raman spectra were subsequently collected from three locations within the bacterial colony area (Fig. 5d). The obtained spectra showed a significant agar contribution, which can be treated by using low-background growth substrate and reducing spectrometer slit width to improve confocality.

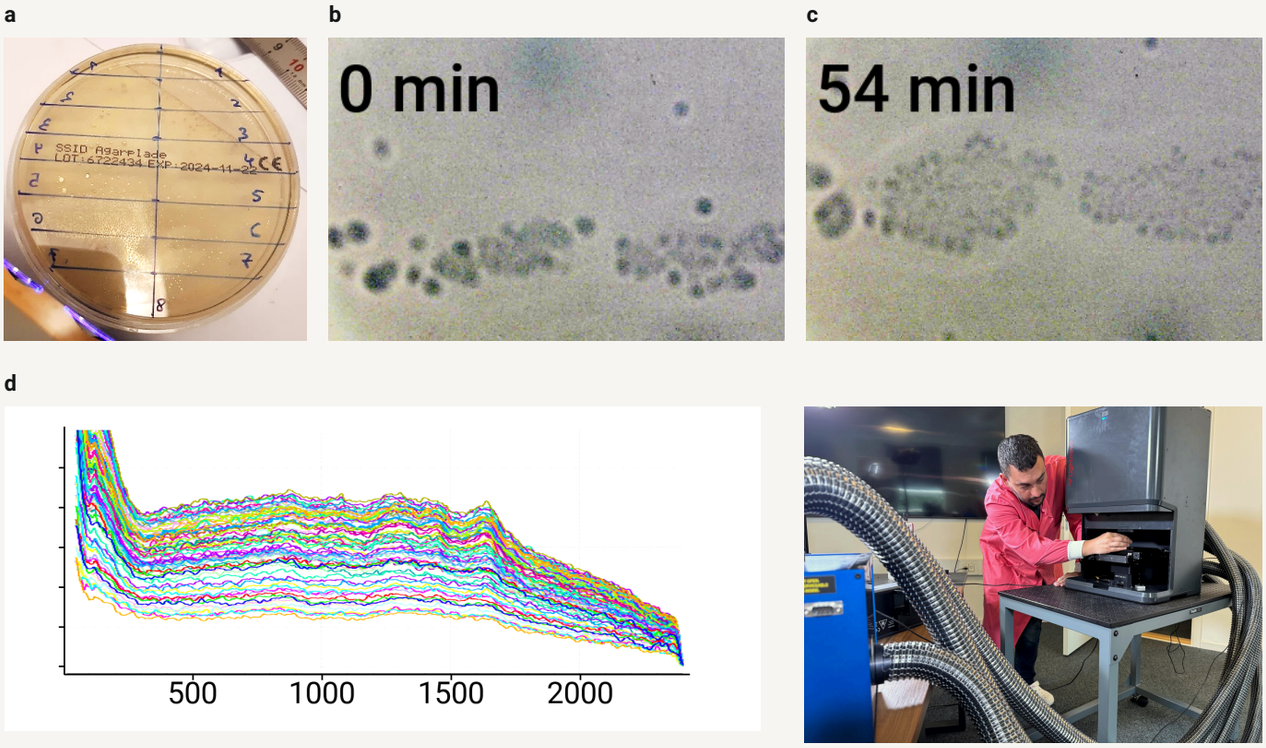


Figure 5. In situ cultivation of *E. coli* inside the RG microscope. (a) Agar plate inoculated with *E. coli* in a streaking pattern (lines 1–8). (b, c) The same field of view at 0 and 54 min of cultivation, showing visible bacterial growth. (d) Raw Raman spectra acquired over a grid on the *E. coli* colony area after 54 min of cultivation (785 nm excitation).

Figure 6. RG microscope with enclosed body and external RG+ air temperature stabilizer (~37 °C, 0.1 °C precision).

04 / WOUND ANALYSIS

Directly from the swab

91 %

accuracy / 20 of 22 held-out maps
12 patients; map-level validation

Infected and immunological wounds are distinguished with 91 % accuracy directly from swabs, without cultivation. Raman spectroscopy offers potential for rapid on-site wound diagnostics, helping clinicians choose between antibiotic and immune-targeted treatment while awaiting laboratory results (up to 3 days).

To evaluate this approach, wound swabs from 12 patients (Odense University Hospital) were resuspended in water and 10 µL dried on a target plate (Fig. 7a, b). A total of 67 Raman maps (8 × 8 points, 785 nm, 5 s per spectrum; Fig. 7c) were acquired. MALDI-TOF analysis of parallel cultures provided reference labels: wounds containing only *S. aureus* and/or *E. coli* were classified as immunological, and the others as infected.

PLS + SVM correctly classified 20 of 22 held-out maps (91 %), with no immunological wounds misclassified as infected (Fig. 7d) [6]. However, 22 % of infected wound maps were classified as immunological. The approach provides results in ~1 h versus 18–72 h for conventional cultivation.

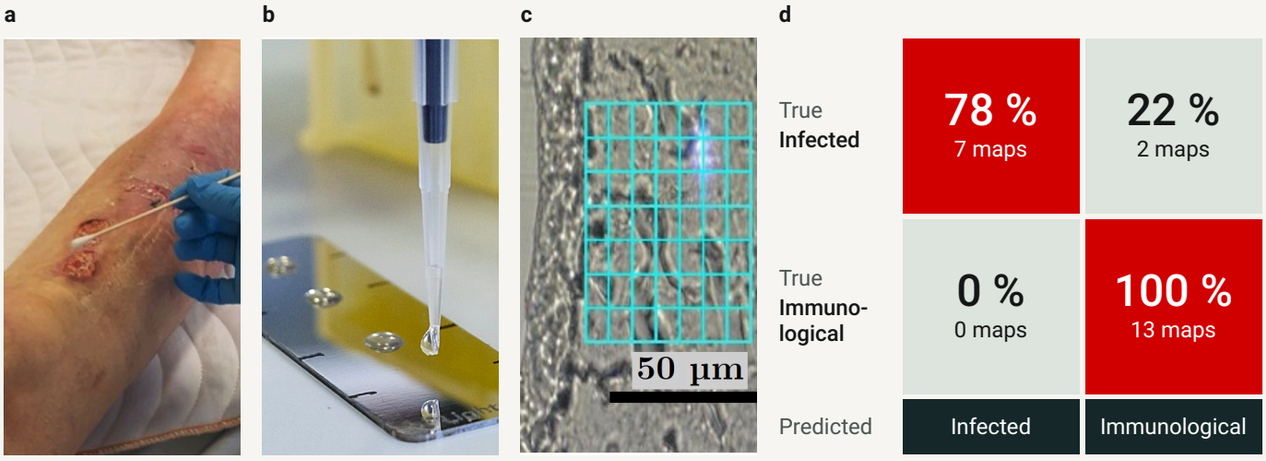


Figure 7. (a) Wound material sampled with a cotton swab. (b) 10 µL of resuspended material deposited on the target plate. (c) 8 × 8 Raman map on the dried material. (d) Infected vs immunological wound, PLS + SVM, map-level confusion matrix for the 22 held-out maps (majority vote of spectra per map); 20 of 22 maps correct (91 %).

Interpretation
This is map-level validation in 12 patients. Labels came from parallel-culture species results; generalisation to unseen patients remains to be validated.

05 / URINE ANALYSIS

Three sampling methods One patient-level comparison

81 %

accuracy / 17 of 21 patients
91 % sensitivity; 70 % specificity

Fig. 9b

	Infected	Not infected
True **Infected**	91 % 10 patients	9 % 1 patient
True **Not infected**	30 % 3 patients	70 % 7 patients
Predicted		

Infected urine is detected with 81 % accuracy (17 of 21 patients) directly from the sample, without cultivation. To identify the most sensitive measurement approach, urine samples were analyzed as dried drops and in liquid form using a contact probe or a quartz cuvette (Fig. 8).

The cuvette measurements were performed at 830 nm, collecting 60 spectra per patient from 21 patients (11 infected, 10 non-infected). After SNR and outlier filtering, PCA + SVM was evaluated using leave-one-patient-out validation with majority voting per patient (Fig. 9a, b).

The cuvette achieved 81 % accuracy, with 91 % sensitivity (10 of 11 infected patients) and 70 % specificity (7 of 10 non-infected patients), outperforming dried drops (65 %) and the contact probe (58 %) (Fig. 9c). While conventional urine culture requires ≥ 24 h [7], liquid-phase Raman spectroscopy offers potential for preliminary screening within a minute to guide further diagnostic testing.

a b c

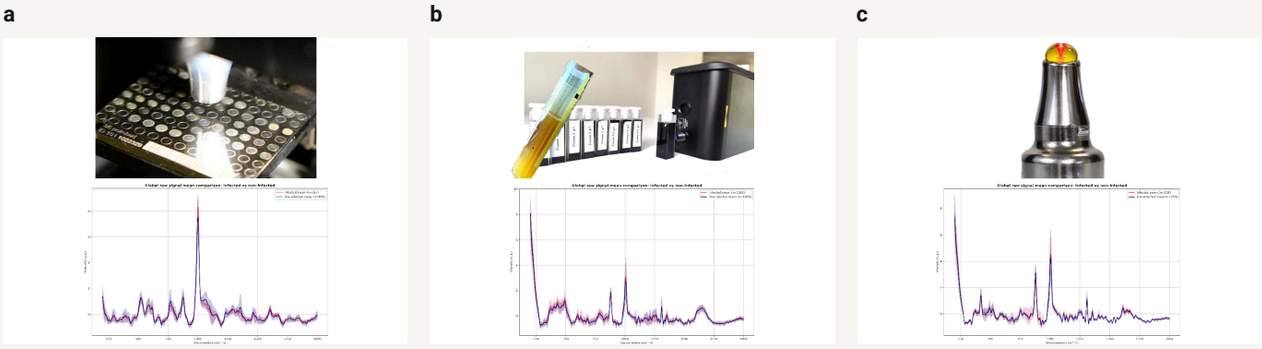


Figure 8. Sampling methods with mean spectra of infected (red) and non-infected (blue) urine: (a) dried drops on the target plate, (b) quartz cuvette, (c) contact probe.

Fig. 9a

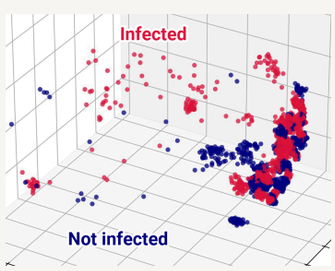


Fig. 9c

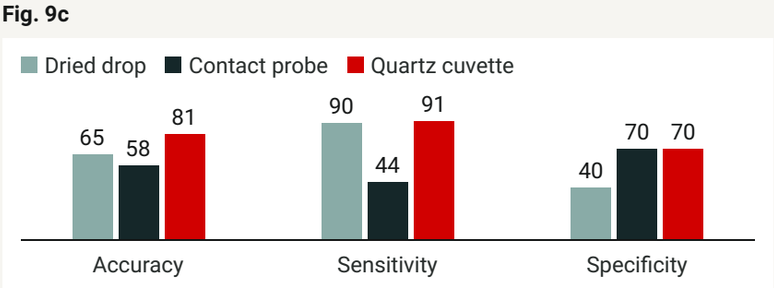
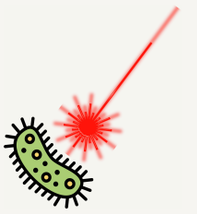


Figure 9. (a) PCA of urine Raman spectra measured in a quartz cuvette (red: infected, blue: non-infected). (b) PCA + SVM classification with leave-one-patient-out validation and majority voting (21 patients, 81 % accuracy). (c) Patient-level accuracy, sensitivity and specificity (%) of the three sampling methods.

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THE RESULTS IN CONTEXT

Five use cases
A path to faster answers



After cultivation	15 species identified from colonies with 97 % accuracy (66 of 68, leave-one-map-out); 88–93 % on unseen measurement days.
Throughput	Up to 49 samples per target plate measured and classified automatically in < 1 h (60 s per sample).
In the instrument	*E. coli* micro-colony growth followed on the stage at 37 °C within 54 min.
Without cultivation	Infected vs immunological wound swabs 91 % (20 of 22 held-out maps, leave-one-map-out); infected urine 81 % with 91 % sensitivity, leave-one-patient-out.
Next	Validation on unseen patients, identification from early micro-colonies, and antibiotic susceptibility testing.

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